

The diagram illustrates the steps of cDNA library construction:

- RNA**: The starting material, represented by a wavy line with 5' and 3' ends. The 3' end is labeled **AAA**.
- 1st strand cDNA**: Formed by adding **oligo dT-T<sub>7</sub> (100 pmol)** and **Reverse transcriptase**. The resulting structure shows the RNA template (5' to 3') paired with the first cDNA strand (3' to 5'), which has a **TTT-T<sub>7</sub>** sequence at its 5' end.
- 1st strand cDNA**: The RNA is removed by **99°C 5'** **denaturing to separate RNA/DNA complex**. The remaining first cDNA strand is 3' to 5' with the **TTT-T<sub>7</sub>** sequence at the 5' end.
- ds cDNA**: A second strand is synthesized using a **random hexamer (100 ng)**, **Klenow**, and **T<sub>7</sub> DNA Polymerase** at **37°C 2 hr**. The second strand is 5' to 3' and starts with a **5' primer**. The double-stranded cDNA (ds cDNA) structure shows the first strand (3' to 5', **TTT-T<sub>7</sub>** at 5') and the second strand (5' to 3', **AAA-T<sub>7</sub>** at 3').
- cRNA**: Produced by **In Vitro Transcription** using **T<sub>7</sub> RNA Polymerase** and **biotinylated CTP/UTP**. The final product consists of three wavy lines representing cRNA molecules, each with a 3' end and a 5' end labeled **UUU**.

FIGURE 1